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(54) Title: USE OF TRANSFORMING GROWTH FACTOR β AND GROWTH FACTORS IN THE TREATMENT AND PREVENTION OF DISEASES OF THE INTESTINAL MUCOSA

(57) Abstract: The present invention relates to the use of transforming growth factor β(TGF-β) and anabolic growth factors (AGF) in the treatment and/or prevention of malfunction or disease of the intestinal mucosa. More in particular the invention relates to the treatment and/or prevention of damage of the intestinal mucosa as a result of chemotherapy of radiotherapy or of inflammatory bowel diseases with a product comprising: a) a first pharmaceutical composition comprising TGF-β in the substantial absence of insulin-like growth factor-1(IGF-1); b) a second pharmaceutical composition comprising AGF in the substantial absence of TGF-β;

USE OF TGF BETA AND GROWTH FACTORS IN THE TREATMENT AND PREVENTION OF DISEASES OF THE INTESTINAL MUCOSA

The present invention relates to the use of a composition containing transforming growth factor β (TGF-β) and a composition containing anabolic growth factors (AGF), in particular insulin-like growth factor 1 (IGF-1) for the prevention or treatment of malfunction or disease of the intestinal mucosa. The invention further relates to a composition containing TGF-β and specific fibres and/or immunoglobulines and/or calcium which can also be used for such a treatment, in particular in combination with IGF-1. The composition containing TGF-β is administered during a period in which it is desired to inhibit cell proliferation and stimulate cell differentiation. The composition containing IGF-1 is administered to restore intestinal epithelial cells.

TGF-β is a multifunctional protein found in all mammalian tissues. Currently, five forms of TGF-β are known, β1 to β5. It has been implicated in the development, differentiation and growth of tissue and the control of immune system function and carcinogenesis. TGF-β can be isolated from natural sources (e.g. blood platelets), mammalian milk or colostrum or can be produced by recombinant cells.

IGF-1 is a small protein (molecular weight about 7800) which plays an important role in bone metabolism. It has been shown to stimulate growth of cells in culture. Animal growth is also stimulated in pituitary deficient, normal and catabolic states. Kidney function is also improved. It can be produced using recombinant DNA technology, solid phase peptide synthesis, by isolating it from blood serum or from human or bovine milk.

TGF-β and its uses are for instance described in EP 852913. This document relates to an enteral food preparation which contains casein rich in TGF-β2, a lipid source such as medium chain or long chain triglycerides or polyunsaturated fatty acids and a carbohydrate source, i.e. maltodextrin, corn starch or sucrose. This composition is used in the treatment or prophylaxis of inflammatory conditions of the gastrointestinal tract, such as Crohn's disease.

EP 462,398 describes the combination of TGF-β1 and a polyunsaturated fatty acid (PUFA) such as linoleic acid, alpha-linolenic acid, gamma-linolenic acid, arachidonic acid, dihomo-

gamma-linolenic acid, eicosapentaenoic acid and/or docosahexanoic acid and/or a derivative thereof for treatment of neoplastic diseases.

WO 96/34614 describes a method for preventing and/or treating damage to the lining of the alimentary tract resulting from chemotherapy and/or radiation, wherein a milk product extract including a mixture of cell growth factors is administered to a patient. The milk product extract, preferably a cheese whey extract, may contain lactoferrin and lactoperoxidase and it can be supplemented with growth factors such as IGF-1, IGF-2, TGF-β, TGF-α, EGF, PDGF, FGF or KGF. This document does not mention which of the substances mentioned should be present in order to achieve the desired preventing or curing effect.

In an article of S.T. Sonis et al, Cancer Res. 54:1135-1138 (1994); "Prevention of chemotherapy induced ulcerative mucositis by transforming growth factor β3" it is described that TGF-β3 administration reduced proliferation of oral epithelium in vitro and in vivo. Topical application of TGF-β3 to the oral mucosa of the Syrian golden hamster prior to chemotherapy significantly reduced the incidence, severity and duration of oral mucositis, reduced chemotherapy associated weight loss and increased survival. Prevention of mucositis according to this document is based on limiting the rate of basal epithelial cell proliferation by prior administration of a negative growth regulator.

It was found according to the invention that for optimum protection of the intestinal mucosa against the damaging effect of chemotherapy and radiotherapy TGF-β should be administered without the presence of IGF-1, in particular any anabolic growth factor, during the chemotherapy or radiotherapy. It was furthermore found that after the chemotherapy or radiotherapy, damaging effects that may have occurred during this therapy can be treated by administering anabolic growth factors, in particular IGF-1 in the substantial absence of TGF-β. According to the invention it was also found that the same sequential administration of TGF-β and IGF-1 can be beneficial in case of inflammatory bowel diseases (IBD), such as Crohn's disease.

The present invention therefore provides the use of TGF-β and AGF in the preparation of a product for use in the treatment and/or prevention of malfunction or disease of the intestinal mucosa; the product comprising:

a) a first pharmaceutical composition comprising TGF-β in the substantial absence of IGF-1;
b) a second pharmaceutical composition comprising AGF in the substantial absence of TGF-β;

wherein the first and second composition are administered sequentially.

Preferably, the weight ratio TGF-β/IGF-1 in the first pharmaceutical composition is at least 100.

It has been found that when a mixture of growth factors is used, for instance when a milk product extract is used, the beneficial effect of TGF-β is reduced by the presence of anabolic growth factors. Therefore, it is preferred to administer TGF-β in the substantial absence of such growth factors.

According to a preferred embodiment of the invention the first pharmaceutical composition comprises TGF-β in substantial absence of AGF. With AGF any anabolic growth factor is meant, i.e any growth factor that would promote cell growth. Examples thereof are: IGF-1, insulin-like growth factor 2 (IGF-2), growth hormone, epidermal growth factor (EGF), transforming growth factor α (TGF-α), mammalian milk growth factor (MMGF = Beta-cellulin) and fibroblast growth factor (FGF). EGF is for instance described in EP 0546 068, MMGF in WO 99/24470. The ratio TGF-β/AGF is preferably at least 50.

Preferably, the AGF in the second pharmaceutical composition is IGF-1. This means that preferably at least IGF-1 is present in the second composition.

According to the present invention when growth factors are mentioned, these include also the active peptide analogues of these growth factors. With peptide analogue is meant any peptide having substantially the same activity as the growth factor, particularly any peptide analogue which is 90% or more homologous with the growth factor.

"Pharmaceutical composition" according to the present invention is meant to include any conventional pharmaceutical preparation such as a capsule, a tablet etc., as well as dietetic preparations such as feed supplements or total feeds.

The sequential administration of the first pharmaceutical composition containing TGF-β and the second pharmaceutical composition containing AGF, preferably at least IGF-1, according to the invention is particular suitable for intestinal disorders in which two phases can be distinguished. The first phase is a phase in which it is desired to inhibit the metabolism. During the second phase, which follows the first phase, the intestinal epithelial cells need to be restored. The composition containing TGF-β is administered during the first phase, the composition containing anabolic growth factors, in particular IGF-1 during the second phase.

More in particular, the sequential administration of TGF-β and IGF-1 is used for the prevention and/or treatment of damage of the intestinal mucosa as a result of chemotherapy and/or radiotherapy. By "damage" is meant any alteration in normal structure or function. Such damage includes mucositis, at least partial loss of mucosal crypt area and/or mucosal villus length, or an increase in bacterial translocation across the alimentary tract.

Chemotherapy and/or radiotherapy are effective at destroying tumours because they target fast-growing tissues. While tumour cells are selectively targeted by anticancer treatments the fast growing tissues of the host are also susceptible, particularly the immune cells of the body and the lining of the alimentary tract. This can result in damage to the linings of the mouth and oesophagus (mucositis, also referred to as stomatitis) and damage to the intestinal lining, commonly in the small bowel and less frequently in the large bowel, leading to severe diarrhoea and pain.

It was found according to the invention that for optimum protection of the intestinal mucosa against the damaging effect of chemotherapy and radiotherapy a first composition containing TGF-β should be administered without the presence of IGF-I, preferably any anabolic growth factor, during the chemotherapy or radiotherapy, in particular during at least the period starting at the latest the first day of said chemotherapy or radiotherapy treatment and ending at the latest the last day of treatment. It was furthermore found that after the chemotherapy or radiotherapy, damaging effects that may have occurred during these therapies can be treated by administering a second composition containing AGF, in particular IGF-1, in the substantial absence of TGF-β.

According to a further embodiment of the invention the sequential administration of TGF-β and IGF-1 is used in the prevention and/or treatment of inflammatory conditions of the intestine, in particular inflammatory bowel diseases (IBD), such as Crohn's disease.

As the TGF-β to be used according to the present invention, every presently available TGF β- can be used e.g. TGF-β1 to TGF-β5. The TGF-β can be of both human and animal origin. Examples thereof are TGF-β which is produced by recombinant cells, TGF-β extracted from blood platelets and TGF-β extracted from milk or whey. Preferably a TGF-β extracted from a mammalian milk product, in particular bovine milk or whey is used because of the reluctance against products obtained by recombinant techniques, cost effectiveness and the presence of other beneficial components in milk or whey extract, such as immunoglobulins. A process for extracting such a TGF-β is described in a copending application of the applicants.

A TGF-β obtained from bovine whey or milk will in general contain more than 100, preferably more than 700 μg TGF-β per g protein. Such an extract will for instance contain 750 μg TGF-β/g protein. The IGF-1 content in this extract will be less than 4, preferably less than 1 μg/g protein.

Preferably the TGF-β is present in the composition in such an amount that 50 ng to 150 μg per day is administered. In case of a liquid product, this will contain TGF-β in a concentration of 0.5 μg -1.5 mg TGF-β per litre. The patient will be administered about 100 ml per day of such a liquid product.

It is preferred that the first pharmaceutical composition according to the invention also contains fibres which upon fermentation form more than 15 g of butyrate per 100 g of short chain fatty acids, preferably more than 20 g of butyrate per 100 g of short chain fatty acids. This characteristic means that the composition should contain fibres that release a relative large amount of butyrate when they are fermented in the intestine (colon). The amount of butyrate can be determined by the method described in Journal of Clinical Nutrition, 1991, no. 53, p. 1418-1424.

Certain disorders of gut function, for instance resulting from chemotherapy can influence the intestinal flora, which causes a temporary decrease of the fermentation to butyrate, in par-

ticular in those cases that the patient is also given a large amount of antibiotics. It is therefore important to administer fibres to the patient which stimulate the synthesis of butyrate by bacteria whereby more butyrate is released into the intestine. Butyrate is a preferential energy substrate in certain intestinal cells, and it also inhibits proliferation and increases differentiation of these cells.

If butyrate is administered as its free salt, undesirable off-flavours can occur. Further only part of the butyrate would reach the colon. A sustained release preparation could overcome this problem, however, these preparations are relatively expensive.

Therefore, according to the present invention it is proposed to administer specific fibres which upon fermentation result in butyrate. Such fibres are: resistant starch, oats bran, in particular the arabinoxylan rich fraction that is poor in β-glucan, some soy fibre extracts and wheat bran. Preferably, wheat bran is used. The amount of fibre is such that a daily ratio of 1 to 30 g, preferably 3 to 10 g, is obtained. In a liquid preparation the concentration is thus 10 to 300 g/l.

The TGF-β composition according to the invention preferably contains immunoglobulins, more in particular in combination with the above mentioned fibres. Their main function is to interact with harmful micro-organisms such as bacteria. This prevents the micro-organism from entering the blood circulation system. This situation in particular occurs when the intestinal mucosa of the patient has been damaged as a result of treatment with chemotherapy.

The immunoglobulins can be isolated from milk of mammals which have been hyperimmunised against certain pathogens or they can be isolated from normal bovine whey or milk. With the process described in the above mentioned patent application, using normal cow's milk as a starting material, a preparation is obtained rich in IgG and IgA. 30 to 50 % of the protein fraction consists of immunoglobulins of the type IgG and IgA. The concentration immunoglobulins in the preparation will, in case of a liquid preparation of 100 ml, be 0.1 to 1500 mg/l.

According to a further preferred embodiment the TGF-β composition contains calcium, preferably in combination with the above mentioned fibres, more preferably in combination with said fibres and immunoglobulins. The calcium can be in the form of finely dispersed

calcium phosphate, calcium carbonate, calcium citrate or a calcium concentrate from bovine milk. The addition of calcium reduces the risk of infection. Calcium lowers the proliferation rate of the epithelial cells. The amount of calcium is more than 50 mg/100 ml, preferably more than 100 mg/100 ml, for instances 120 mg/100 ml, based on a liquid composition.

It has been found that when a product containing TGF-β, butyrate producing fibres and high levels of calcium salts is administered a synergistic effect occurs resulting in a composition that is effective in preventing damage to epithelial gut cells during chemotherapy and radiotherapy and in the treatment of inflammatory bowel diseases.

Preferably, the first pharmaceutical composition containing TGF-β according to the invention also contains one or more of the following ingredients: proteins, fat, minerals, trace elements, vitamins, fatty acids and lactoferrin.

Preferably, proteins are present in an amount of 3 to 10 % protein equivalents, this includes intact protein, peptides and amino acids. The amount of fat is preferably 2 to 10 %, based on the total weight of the preparation. The amount of minerals, trace elements and vitamins is according to the daily recommended dosage.

Preferred vitamins are vitamin A, C and E. Vitamin A and provitamin A are required. Their concentration is preferably more than 130 μgRE/100 ml, in particular more than 300 μg/100 ml. Suitably part of the vitamin A is administered as retinoic acid or a metabolic equivalent thereof. Vitamin C and tocopherols are administered because of their role in the antioxidant cascade. During radiotherapy but also with initial inflammatory reactions they can protect the epithelial cells. The concentration vitamin C or an equivalent thereof is more than 40 mg/100 ml, preferably more than 60 mg/100 ml. The concentration of tocopherols is more than 5 mg, preferably more than 15 mg/100 ml.

The fats should provide sufficient fatty acids. Preferably stearidonic acid (STA) is added. Suitable fatty acids and the amounts and ratios in which they are used are described in PCT/EP98/08409, i.e. the fatty acids gamma-linolenic acid, stearidonic acid and eicosapentaenoic acid together constitute 10 to 500 mg/g of the total amount of fatty acids and

gamma-linolenic acid and eicosapentaenoic constitute 20 to 50 wt.% and stearidonic acid forms 15 to 50 wt.% of these three fatty acids.

Lactoferrin can be present because it has anti-bacterial activity against a number of pathogens. This substance can also have a modulating action with initial inflammatory reactions, which are delayed. It is desired to have a daily doses of 0.1 to 3 g of lactoferrin.

It is preferred that the composition contains less than 11 %, preferably less than 6 % digestible carbohydrates. A higher percentage of these substances would affect the taste of the composition. Generally about 4.5 g/100 ml are used. As a source of digestible carbohydrates sucrose, but also slowly digestible carbohydrates can be used.

In the second composition, the dosage of IGF-1 is preferably 0.1 to 100 μg IGF-1 per kg body weight per day. In a liquid product this concentration is 7 μg to 7 mg IGF-1 per 100 ml. The second composition preferably contains substantially no TGF-β. The ratio IGF-1/TGF-β is at least 50, preferably at least 100.

The second composition can further contain immunoglobulins. In view of the severity of the mucositis which has developed, it is important to prevent and/or treat translocation of harmful substances, for instance micro-organisms. Preferably doses of 0.03 mg to 5 mg immunoglobulins per day are administered. If the IGF-1 is obtained from bovine milk, generally a preparation will be obtained containing 10 to 1000 mg Ig per 100 μg IGF-1.

Beside immunoglobulins, fibres can be present. As this composition is administered during a phase wherein the intestinal flora of the patient is extremely disrupted, a mixture of fibres is preferably administered. Preferably theses fibres are soluble non-starch polysaccharides, such as gum arabic or pectin, insoluble non-starch polysaccharides, such as cellulose and hemicellulose and oligosaccharides and/or resistant starch and/or lignin. An example of such a mixture is described in EP 0756828, which is incorporated by reference.

The second composition can further contain one or more of lactoferrin, glutamine and anti-oxidants. Glutamine must have a stable form. In a liquid product, glutamine rich peptides should be used or extracts from hydrolysates of glutamine rich proteins. The amounts of

lactoferrin and antioxidants are the same as in the first composition. Further the second composition may contain fat, protein and other microcomponents, such as minerals, vitamins and trace elements. Further, substances that support the total methionin metabolism can be present.

According to a further embodiment of the invention TGF-β in the substantial absence of insulin-like growth factor 1 IGF-1, in particular in the absence of AGF, and fibres which upon fermentation form more than 15 g of butyrate per 100g of short chain fatty acids and/or immunoglobulins are used for preparing a pharmaceutical composition for treatment and/or prevention of malfunction or disease of the intestinal mucosa, more in particular for treatment and/or prevention of damage of the intestinal mucosa as a result of chemotherapy or radiotherapy or for treatment and/or prevention of inflammatory bowel diseases.

The present invention also relates to a pharmaceutical composition containing TGF-β in the substantial absence of IGF-1, in particular in the absence of AGF, preferably in combination with fibres which upon fermentation form more than 15 g of butyrate per 100 g of short chain fatty acids and/or immunoglobulins.

In case of a liquid composition, such a composition contains per 100 ml

a) 50 ng to 150 µg TGF-β
b) 1 to 30 g fibres
c) 0.01 to 150 mg immunoglobulins
d) 0.03 to 1 g lactoferrin
e) > 50 mg calcium
f) fatty acids
g) > 130 µg RE vitamin A
h) > 40 mg vitamin C
i) > 5 mg tocopherols
j) 3 to 10% protein equivalents

For example, a suitable liquid TGF-β based formula contains per 100 ml:

a) 4 µg TGF-β
b) 5 g wheat bran

c) 2 mg immunoglobulins
d) 0.5 g lactoferrin
e) 80 mg calcium
f) 4 g fat blend containing 30 % MCT, 26 % palm oil, 16 % soy oil, 8 % borage oil, 11 % echium oil, 6.5 % fish oil and 2.5 % egg lipids
g) 300 μg vitamin A
h) 70 mg vitamin C
i) 15 mg α-tocopherol
j) 4 g casein
k) 5 g maltodextrin

Of this formula, 250 ml per day is administered.

The invention also relates to a pharmaceutical composition containing AGF, preferably IGF-1, in the substantial absence of TGF-β and fibres selected from soluble non-starch polysaccharides, such as gum arabic or pectin, insoluble non-starch polysaccharides, such as cellulose and hemicellulose and oligosaccharides and/or resistant starch and/or lignin. The composition preferably further contains at least one member of the group comprising lactoferrin, glutamine and antioxidants.

In case of a liquid composition, such a composition contains per 100 ml:

a) 7 μg to 7 mg IGF-1
b) 1 to 30 g fibres
c) 5 to 300 mg immunoglobulins
d) 0.3 to 3 g lactoferrin
e) 0.5 to 10 g glutamine
f) > 130 μg RE vitamin A
g) > 40 mg vitamin C
h) > 5 mg tocopherols

For example, a suitable liquid IGF-1 based formula contains per 100 ml:

a) 100 μg IGF-1
b) 5 g fibre mix: 1g wheat bran, 3 g inulin, 1 g oats bran
c) 200 mg immunoglobulins

d) 0.5 g bovine lactoferrin
e) 5 g alanylglutamine
f) 300 µg vitamin A
g) 70 mg vitamin C
h) 15 mg α-tocopherol

Of this formula, 250 ml per day is administered.

The compositions according to the invention can have the form of any oral preparation, for instance capsules, sachets or tablets each containing a predetermined amount of the active ingredient; powders or granules; solutions or suspensions in an aqueous or non-aqueous liquid. Preferred dosage forms are food supplements or total feeds or powders which upon reconstitution with a liquid such as water give a total feed or food supplement. The present invention also relates to tube feeds containing these ingredients.

The present invention also relates to products consisting of a combination of the first composition and the second composition for sequential administration for preventing and/or treating damage of the intestinal mucosa as a result of chemotherapy or radiotherapy or for preventing and/or treating inflammatory conditions of the intestine, in particular Crohn's disease.

Claims

1. Use of transforming growth factor β (TGF-β) and anabolic growth factors (AGF) in the preparation of a product for use in the treatment and/or prevention of malfunction or disease of the intestinal mucosa; the product comprising

a) a first pharmaceutical composition comprising TGF-β in the substantial absence of insulin-like growth factor-1 (IGF-1);

b) a second pharmaceutical composition comprising AGF in the substantial absence of TGF-β;

wherein the first and second composition are administered sequentially.

2. Use according to claim 1, wherein the first pharmaceutical composition comprises TGF-β in substantial absence of AGF.

3. Use according to claim 1 or 2, wherein the second pharmaceutical composition comprises at least IGF-1 as the AGF.

4. Use according to any of claims 1 to 3, for the preparation of a product for use in the treatment and/or prevention of damage of the intestinal mucosa as a result of chemotherapy or radiotherapy.

5. Use according to claim 4, wherein the first pharmaceutical composition is administered during the period starting at the latest the first day of said chemotherapy or radiotherapy treatment and ending at the latest at the effective end of said treatment.

6. Use according to any of claims 1 to 3, for the preparation of a product for use in the treatment and/or prevention of inflammatory bowel diseases.

7. Use according to any of claims 1 to 6, wherein the TGF-β is obtained by extraction from a mammalian milk product, preferably bovine milk or whey.

8. Use according to any of claims 1 to 7, wherein the first pharmaceutical composition further contains fibres which upon fermentation form more than 15 g of butyrate per 100 g of short chain fatty acids and/or immunoglobulins and/or calcium.

9. Use according to claim 8, wherein the first pharmaceutical composition contains fibres which upon fermentation form more than 15 g of butyrate per 100 g of short chain fatty acids as well as immunoglobulins and calcium.

10. Use according to claim 8 or 9, wherein the fibres are wheat bran fibres.

11. Use according to any of claims 1 to 10, wherein the first pharmaceutical composition further contains at least one member of the group of lactoferrin, fatty acids and antioxidants.

12. Use according to any of claims 1 to 11, wherein the second pharmaceutical composition further contains fibres selected from the group of soluble non-starch polysaccharides, insoluble non-starch polysaccharides, oligosaccharides, resistant starch and mixtures thereof.

13. Use according to any of claims 1 to 12, wherein the second pharmaceutical composition further contains at least one member of the group comprising lactoferrin, glutamine and antioxidants.

14. Product containing

a) transforming growth factor β (TGF-β) in substantial absence of insulin-like growth factor 1 (IGF-1) and

b) anabolic growth factors (AGF) in substantial absence of TGF-β;

as combination for sequential administration for treating and/or preventing malfunction or disease of the intestinal mucosa.

15. Pharmaceutical composition containing

a) transforming growth factor β (TGF-β) in the substantial absence of insulin-like growth factor 1 (IGF-1) and

b) fibres which upon fermentation form more than 15 g of butyrate per 100 g of short chain fatty acids and/or

c) immunoglobulins and/or

d) calcium.

16. Pharmaceutical composition according to claim 15, which comprises TGF-β in the substantial absence of anabolic growth factors (AGF).

17. Pharmaceutical composition according to claim 15 or 16, containing fibres as well as immunoglobulins and calcium.

18. Pharmaceutical composition according to any of claims 15 to 17, containing fibres in such an amount that 1 to 30 g of fibres per day are administered.

19. Pharmaceutical composition according to any of claims 15 to 18, wherein the fibres are wheat bran fibres.

20. Pharmaceutical composition according to any of claims 15 to 19, wherein the TGF-β is obtained by extraction from a mammalian milk product, preferably bovine milk or whey.

21. Pharmaceutical composition according to any of claims 15 to 20, containing TGF β in such an amount that 50 ng to 150 μg TGF-β per day is administered.

22. Pharmaceutical composition according to any of claims 15 to 21, further containing at least one member of lactoferrin, fatty acids and antioxidants.

23. Use of

a) transforming growth factor β (TGF-β) in the substantial absence of insulin-like growth factor 1 (IGF-1) and

b) fibres which upon fermentation form more than 15 g of butyrate per 100g of short chain fatty acids and/or

c) immunoglobulins and/or

d) calcium

for preparing a pharmaceutical composition for treatment and/or prevention of malfunction or disease of the intestinal mucosa.

24. Use according to claim 23, wherein TGF-β is applied in the substantial absence of anabolic growth factors (AGF).

25. Use according to claim 23 or 24, for preparing a pharmaceutical composition for treatment and/or prevention of damage of the intestinal mucosa as a result of chemotherapy or radiotherapy.

26. Use according to claim 23 or 24, for preparing a pharmaceutical composition for treatment and/or prevention of inflammatory bowel diseases.

27. Pharmaceutical composition containing

a) anabolic growth factors (AGF) in the substantial absence of transforming growth factor-β (TGF-β) and

b) fibres selected from the group of soluble non-starch polysaccharides, insoluble non-starch polysaccharides, oligosaccharides, resistant starch and mixtures thereof.

28. Pharmaceutical composition according to claim 27, containing at least insulin-like growth factor-1 (IGF-1) as the AGF.

29. Pharmaceutical composition according to claim 27 or 28, further containing at least one member of the group comprising lactoferrin, glutamine and antioxidants.

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/NL 99/00620

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/18 A61K38/30 A23L1/30 //(A61K38/30,38:18), (A61K38/18,31:20,31:715,38:40,39:395),(A61K38/30,31:715,38:05, 38:40,39:395)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, FSTA, MEDLINE, CANCERLIT, AIDSLINE, LIFESCIENCES, CHEM ABS Data, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 824 297 A (IWATA K K ET AL) 20 October 1998 (1998-10-20) claims 1-12,27-29; examples 2-7B column 2, line 6 - line 10 column 6, line 25 -column 7, line 16	1-5,14
A	the whole document & US 5 817 625 A (HALEY JOHN DOUGLAS) 6 October 1998 (1998-10-06) column 2, line 23 - line 36	15-26
A	BECK P L & WALLACE J L: "Cytokines in inflammatory bowel disease." MEDIATORS OF INFLAMMATION, vol. 6, no. 2, April 1997 (1997-04), pages 95-103, XP000929627 table 2	6

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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2 October 2000	06. 10. 2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Teyssier, B

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intern. ..al Application No
PCT/NL 99/00620

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT

International application No.
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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

In claims 11, 13, 22 and 29 the designation "antioxydants" relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only two such compounds: Vitamins C and E (alpha-tocopherol). In the present case the claims so lack support and the application so lacks disclosure that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those two compounds which appear to be supported and disclosed and their immediate derivatives.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-14

   Use of TGF-beta and so called "anabolic" growth factors in the preparation of two separate compositions for sequential use in the treatment or prevention of diseases of the intestinal mucosa; product containing the two compositions.

2. Claims: 15-26

   Pharmaceutical composition comprising TGF-beta, butyrate-producing fibers, immunoglobulins, calcium.

3. Claims: 27-29

   Pharmaceutical composition comprising a so called "anabolic" growth factor and fibers.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 99/00620

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(54) METHOD OF UTILIZING BODY TASTE IMPROVER COMPRISING LONG-CHAIN HIGHLY UNSATURATED FATTY ACID AND/OR ESTER THEREOF

(57) The purpose of the invention is to enhance the body taste, taste and flavor of foods.

Thus, the present invention is related to a method for enhancing or making better body taste or taste of the foods by adding n-3 long-chain highly unsaturated fatty acid having 20 or more of carbon atoms and 3 or more of double bonds, or adding n-6 long-chain highly unsaturated fatty acid having 18 or more of carbon atoms and 3 or more of double bonds to them.

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Description

Technical Field

[0001] The present invention relates to a method of application of a body taste (or "kokumi" taste) enhancer comprising a long-chain highly unsaturated fatty acid and/or an ester thereof, more particularly to a method for enhancing or making better flavor or taste of food by means of the long -chain highly unsaturated fatty acid and/or the ester thereof, the body taste enhancer comprising thereof, or a vegetable fat and oil composition comprising thereof.

Backaround of the Invention

[0002] Arachidonic acid (cis-5,8,11,14-eicosatetraenoic acid) belongs to long-chain highly unsaturated (polyunsaturated) fatty acids, and exists in a phospholipid derived from animal organs or tissues. This fatty acid is an essential one, and is very important as a precursor for the synthesis of prostaglandin, thromboxane, leukotriene, etc

[0003] Attempts have been made to add the long-chain highly unsaturated fatty acids such as arachidonic acid and esters thereof for enrichment of nutrition and provision of various physiological functions in view of the above remarkable functions of arachidonic acid.

[0004] An enriched composition described in Japanese Patent Application laid open Hei 10 (1998)-99048 comprises arachidonic acid in an amount of 0.1~10 % by weight as one of the components added for realizing a composition similar to that of mother's milk.

[0005] As an example of the above ester, Japanese Patent Application laid open Hei 4 (1992)-197134 discloses fat and oil composition for frying, which is protected against a decrease in temperature of an inner material due to evaporation latent heat. The composition comprises as constituting fatty acid unsaturated fatty acids such as arachidonic acid in an amount of 20~60 % by weight.

[0006] Japanese Patent Application laid open Hei 9 (1997)-13075 discloses fat and oil consisting of glyceride comprising long-chain highly unsaturated fatty acids such as arachidonic acid, and having a fu nction of reducing the concentration of fatty and oil in blood. The glyceride is obtained by transesterification. It has a different structure from natural one, in which less than 40 mol % of the total long-chain highly unsaturated fatty acids are bound to 2 position of the glyceride.

[0007] Japanese Patent Application laid open Hei 9 (1997)-13076 discloses fat and oil with the same composition as the above one, having a function of inhibiting platelet aggregation.

[0008] Japanese Patent Application laid open Hei 11 (1999)-89513 discloses a synthetic fat and oil composition similar to human milk fat and oil, in which n-6 long-chain highly unsaturated fatty acid such as arachidonic acid, is used as one of the constituting fatty acids of triglyceride.

[0009] Furthermore, Japanese Patent Application laid open Hei 10 (1998)-70992 and Japanese Patent Application laid open Hei 10 (1998)-191886 disclose edible oil derived from microorganism, which has a lot of arachidonic acid in a form of triglyceride. Its preferred application includes modified milk for a premature baby or an infant, food for infant, and food for a pregnant woman.

[0010] However, there is no disclosure of technology about use of the long-chain highly unsaturated fatty acid for the purpose of enhancing taste such as a body taste of foods and vegetable fat and oil, or no description to suggest a possibility to do that.

[0011] There has been a problem that the addition of the long -chain highly unsaturated fatty acid to foods would deteriorate their taste due to odor smell revers ion flavor derived from oxidized decomposition of the fatty acid. Many means have been tried to solve the problem.

[0012] One of the those means is disclosed in the Japanese Patent Application laid open Sho 63 (1988)-44843 wherein a highly unsaturated fatty acid is included in an inner oil phase of an oil-in-water-in-oil-type emulsion composition. The Japanese Patent Application laid open Hei 6 (1994)-172782 discloses technology of pulverizing fat and oil comprising a highly unsaturated fatty acid.

[0013] The Japanese Patent Application laid open Hei 9 (1997)-176679 discloses technology of mixing anti-oxidant powder with a pulverized unsaturated fatty acid. The Japanese Patent Application laid open Hei 9 (1997)-263784 discloses technology of mixing δ-tocopherol with fat and oil comprising a polyunsaturated fatty acid. The Japanese Patent Application laid open Hei 11 (1999)-12592 discloses technology of adding soybean source to fish fat and oil comprising a highly unsaturated fatty acid.

[0014] The Japanese Patent Application laid open 2001-78702 discloses as an example of application of highly unsaturated fatty acids in the field of food a seasoning having enriched mildness, "umami" taste, and after taste, which is prepared by mixing fat and oil with extract into an oil-in-water-type emulsion. Fish oil or fat and oil comprising the fish oil is disclosed as an example of the above fat and oil, including one wherein 10 % by weight or more of the fish oil is made of n-3 (ω-3) highly unsaturated fatty acids.

[0015] It is preferred to use a fatty acid ester of polyglycerine as emulsifier and to use extract wherein an antioxidant such as carnosine and anserine for preventing oxidization of the fat and oil. No oxidization treatment such as heating treatment is not carried out in a process for the reparation of the seasoning. Examples of foods on which the effect of the seasoning is significantly performed include minced products, fish and processed fish products.

[0016] The Japanese Patent No.3220155 discloses a flavoring composition which is prepared by oxidization of fatty acids except milk fat and is characterized by comprising at least one of polyunsaturated fatty acids with n-3 non-conjugated double bond in an amount of more than 0.01 % by weight. This flavoring composition comprises swe et and creamy note that is remarkably recognized in butter-like flavor. In order to obtain such note, the fat and oil need to be subjected to oxidization treatment, which has to be carried out under control during a process in the presence of an anti-oxidant that will slightly delay the oxidization. As the flavor generated in the oxidization treatment contains volatile components, the oxidization treatment is preferred to do in a closed system. Actually, the oxidization treatment is done by using a reflux condenser in an example. It is described that the flavoring composition is particularly suitable in use for adding flavor to foods that are advantageously desired to have butter flavor.

[0017] US Patent No.3,689,289 discloses a method for the production of artificial chicken flavor by heat-reacting reducing sugar, amino acid and arachidonic acid or its methylester under particular conditions. Further, International Publication WO03/051139 pamphlet discloses a method for the production of artificial chicken flavor by heat-reacting reducing sugar, amino acid and arachidonic acid under particular conditions, wherein heat resistance and persistency of the artificial chicken flavor is obtained by using the arachidonic acid in a form of glycerin ester.

[0018] As a product obtained by heating three compounds of the sugar, amino acid and arachidonic acid will give the artificial chicken flavor in these methods, there is no description about enhancement of taste of foods by use of the long-chain highly unsaturated fatty acid such as arachidonic acid alone, or in a combination with the amino acid or sugar.

[0019] Japanese Patent Application laid open 2002-95439 discloses seasoning comprising glyceride of a long-chain highly unsaturated fatty acid, which enables one to take the long-chain highly unsaturated fatty acid together with a wide range of foods. The purpose of this invention is to increase oxidation stability of the long-chain highly unsaturated fatty acid that is susceptible to deterioration due to oxidation. Accordingly, it is characterized by adding the glyceride of the long-chain highly unsaturated fatty acid to processed foods prepared by fermentation of soybean or fish and shell, or to the seasoning composed mainly of tomato's components. Thus, there is no disclosure or teaching of oxidization treatment of the long-chain highly unsaturated fatty acid, or of the enhancement or provision of body taste of foods by the same acid per se.

[0020] [Patent document 1] Japanese Patent No.3220155
[Patent document 2] US Patent No.3,689,289
[Patent document 3] International Publication WO03/051139 pamphlet
[Patent document 4] Japanese Patent Application laid open 2002-95439

Summary of the Invention

[0021] In the field of food, there are some kinds of foods that require "body taste" and "rich or thick taste", such as fried foods including pork cutlet, fat and oil containing foods including curry source and "gyo-za" (Chinese-style pork dumpling)." Conventionally, flavor has been added, or animal fat and oil have been used alone or in combinati on with vegetable fat and oil in order to give the above tastes to those foods.

[0022] However, there is a problem that the added flavor is volatilized during a heating treatment so that the given body taste can not be maintained. There is also a concern that cholesterol or saturated fatty acids contained in the animal fat and oil may adversely affect health. On the other hand, since the vegetable fat and oil contain a small amount of cholesterol or saturated fatty acids, there is a problem that foods cocked with the vegetable fat and oil would taste simple or plain, making the foods that need body taste unsatisfactory.

[0023] It is therefore desired to provide fat and oil being free of cholesterol and having a low content of saturated fatty acid, while having body taste.

[0024] A long-chain highly unsaturated fatty acid such as arachidonic acid and/or an ester thereof has been considered for a long time a causative agent of putrefactive smell of meat and the like and off-flavor. However, it was found that when foods are mixed with the long-chain highly unsaturated fatty acid and/or the ester thereof alone or are subjected to oxidization treatment such as heating with vegetable fat and oil comprising a predetermined amount of said long-chain highly unsaturated fatty acid and/or the ester thereof, the body taste of the foods will be enhanced and the original tastes of the foods will be increased (PCT/JP03/00182).

[0025] The present inventors has studied to overcome the above problem, and finally found that a long-chain highly unsaturated fatty acid and/or an ester thereof has various functions such as enhancing not only body taste but also taste and flavor of foods when added to the foods.

[0026] Thus, the present invention relates to a method for enhancing or making better taste or flavor of foods, comprising adding a long-chain highly unsaturated fatty acid and/or an ester thereof to the foods.

[0027] The phrase "enhancing or making better taste or flavor of foods" in the present specification means to newly provide a significant "body taste" and "rich or thick" with foods, to enhance them, and to inhibit unfavorable smell or odor that is peculiar to each food. The above advantages of the present invention may be evaluated by a sensory test.

[0028] There are listed below representative aspects of the method for enhancing or making better taste or flavor of foods, comprising adding a long-chain highly unsaturated fatty acid and/or an ester thereof to the foods.

[0029] That is, by the addition of the long -chain highly unsaturated fatty acid and/or the ester thereof;

1. A method for making taste of seasoning better, and/or for providing seasoning with body taste;
2. A method for enhancing body taste of extract;
3. A method for enhancing egg flavor of processed egg food;
4. A method for enhancing body taste of soup;
5. A method for providing curry roux or stew with body taste,;
6. A method for inhibiting heat-browning odor of Japanese soup or its stock;
7. A method for providing processed animal meat food with body taste;
8. A method for enhancing body taste and fried-egg taste of fried rice; and
9. A method for inhibiting proteinous odor of vegetable protein.

[0030] The "long-chain highly unsaturated fatty acid" in the present specification means a fatty acid having 20 or more of carbon atoms and 3 or more of double bonds in the case of n-3 long-chain highly unsaturated fatty acids, and a fatty acid having 18 or more of carbon atoms and 3 or more of double bonds in the case of n-6 long-chain highly unsaturated fatty acids. A long-chain highly unsaturated fatty acid having 20~24 carbon atoms and 4~6 double bonds is preferred in both cases. Examples of the n~6 long-chain highly unsaturated fatty acids include γ-linolenic acid, arachidonic acid (AA) and docosatetraenoic acid (DTA), arachidonic acid being preferable. Examples of the n-3 long-chain highly unsaturated fatty acids include docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).

[0031] There is no limitation on an origin of the long -chain highly unsaturated fatty acid such as arachidonic acid and its ester used in the present invention. Those skilled in the art may optionally use those derived from various animals and vegetables, microorganisms and algae that are commercially available.

[0032] For example, Japanese Patent Applications laid open Hei 10 (1998)-70992 and Hei 10 (1998)-191886 disclose edible fat and oil derived from bacteria, which comprises a lot of arachidonic acid in a form of triglyceride. The arachidonic acid may be therefore obtained form said edible fat and oil. γ-linolenic acid may be obtained from oil of borage, evening primrose, rose hip and Ribes Nigrum

[0033] It is also possible to mix and use together two or more kinds of the long -chain highly unsaturated fatty acids, or the long-chain highly unsaturated fatty acids that have different origins but belong to the same kind.

[0034] There is no limitation on structure and preparation of the ester of the long -chain highly unsaturated fatty acid, and monohydric and polyhydric alcohols may be used as alcohol that constitutes the above ester. Glycerol is one of the preferred examples of the polyhydric alcohols in view of safety and cost. The glycerol will constitute tri-glyceride, di-glyceride or mono-glyceride. Other fatty acids besides the long-chain highly unsaturated fatty acid may be contained as fatty acids that constitute the ester of the present invention.

[0035] In the method of the present invention, the long-chain highly unsaturated fatty acid and/or an ester thereof may be added as such or in a form of a preparation of the body taste enhancer comprising them in order to enhance or make better taste or flavor of foods.

[0036] There is no limitation on a content of the long-chain highly unsaturated fatty acid and its ester comprised in the body taste enhancer according to the present invention. However, if the content is too low, a more amount of in the body taste enhancer shall be needed, which may cause disadvantageous effects due to other components contained therein. The body taste enhancer of the present invention comprises the long -chain highly unsaturated fatty acid and its ester preferably in an amount of 10 % by weight or more, more preferably 30 % by weight or more.

[0037] The body taste enhancer of the present invention may additionally contains other optional components known to those skilled in the art, such as an emulsifier; tocopherols; sterols; phospholipids and other fatty acids; triglycerides, diglycerides and monoglycerides containing the other fatty acids.

It is desirable to subject the n-3 long-chain highly unsaturated fatty acid and/or the ester thereof to oxidization treatment in order to sufficiently effect the advantage. There is no limitation on a method for the oxidization treatment, which, for example, includes heating treatment. There is no limitation on a method for heating treatment, either. It is not necessary to carry out the oxidization treatment in the presence of an antioxidant, or in a closed system. The heating may therefore be normally carried out at 40°C~200°C for 0.1~240 hours, preferably at 80°C~180°C for 0.5~72 hours.

[0038] Although it is not necessary to subject the n-6 long-chain highly unsaturated fatty acid and/or the ester thereof to the oxidization treatment in order to effect its advantage, which shall be further increased by the oxidization treatment. The advantage may be obtained by subjecting the body taste enhancer comprising the long-chain highly unsaturated fatty acid and/or the ester thereof to the oxidization treatment such as heating.

[0039] The long-chain highly unsaturated fatty acid is more volatile than its ester, especially glycerine ester, even a less amount of said acid can effect the advantages of the present invention. On the other hand, as esters are relatively less volatile but persistently effective, they are comprised in the composition in a relatively larger amount.

[0040] Further when the long-chain highly unsaturated fatty acid such as arachidonic acid and/or the ester thereof derived from the mi croorganism is used, it will be possible to obtain the body taste enhancer or foods having substantially no fat and oil derived from animal and a low content of saturated fatty acid and being free of cholesterol.

[0041] There is no limitation on an amount or timing with respect to the addition of the long-chain highly unsaturated fatty acid and/or its ester. IT may be added in an appropriate amount at an appropriate time depending on a kind and way of cooking of the foods. If the foods are heated after the long-chain highly unsaturated fatty acid and/or its ester is added to them, the advantages of the present invention may be more significantly obtained. There is no limitation on the kind of subject foods.

[0042] By adding the long-chain highly unsaturated fatty acid and/or the ester thereof to the foods, it will be possible to enhance or make better taste or flavor of the foods. Coexistence of reducing sugar or amino acid is not necessary to obtain the advantages of the present invention.

Best Mode for Carrying Out the Invention

[0043] The applications of the method of the present invention will be described below more in detail referring to the representative foods.

(1) Seasoning

[0044] The addition of the long-chain highly unsaturated fatty acid and/or the ester thereof to seasoning obtained by mixing one or more of salt, saccharides such as sugar, organic acids such as vinegar, MSG (monosodium glutamate), IN (sodium 5'-inosinate), soy sauce, soybean paste, and proteinous hydrolyate with acid or enzyme will make taste of the seasoning better, and/or will provide it with body taste. There is no specific limitation on a form of the seasoning, including powder, paste, liquid and the like. Powdered fat and oil may be mixed with the seasoning. The powdered fat and oil may be supplemented with various known auxiliaries such as a thickening agent, or be emulsified with an emulsifier. Powdering may be done by any method known to those skilled in the art such as spray-dry or freeze-dry. The thus prepared seasoning may be added to foods so as to enhance their body taste.

(2) Extract(s)

[0045] The addition of the long-chain highly unsaturated fatty acid and/or the ester thereof to extract will enhance body taste of the extract, or will provide it with freshly-prepared (or boiled) flavor and taste. The extract includes fish and shell extract prepared by extraction from seafood such as skipjack tuna, chub mackerel, scallop, oyster, and kelp; animal meat extract prepared by extraction from animal meat such as pork, chicken and beef, and bone, a part of bone and the like of animals; yeast extract extracted from yeast; and vegetable extract prepared by extraction from various vegetables such as onion, garlic, and cabbage. There is no limitation on a concentration of the extract when the long-chain highly unsaturated fatty acid and/or the ester thereof is added to them. There is no limitation on a form of the extract, including power and paste and the like. As already mentioned, there is no need of addition of the reducing sugar or amino acid to the extract to be mixed with the long-chain highly unsaturated fatty acid and/or the ester thereof in order to show the body taste enhancing effect. The long-chain highly unsaturated fatty acid and/or the ester thereof may be added at any step during a production process of the extract.

[0046] Although the long-chain highly unsaturated fatty acid and/or the ester thereof may show its advantages even if it is added after heating of the extract, it may show more effectively its advantages if it is heated after being mixed with the extract. The fat and oil may be removed by filtration and the like after the long-chain highly unsaturated fatty acid and/or the ester thereof is mixed and heated with the extract. The mixture of the long-chain highly unsaturated fatty acid and/or the ester thereof and the extract may be heated at 50°C~130°C, preferably at 80°C~100°C for 10 min~6 hours, preferably for 1~3 hours. The long-chain highly unsaturated fatty acid and/or the ester thereof may be added to the extract usually in an amount of 1~50,000 ppm, preferably of 10~10,000 ppm as of the long-chain highly unsaturated fatty acid. The advantages of the present invention may be obtained even if the extract is subjected to other treatments such as disintegration and drying after being mixed with the long-chain highly unsaturated fatty acid and/or the ester thereof.

[0047] The taste of foods such as curry and soup may be made better, or the foods will be provided with the body taste, by adding to the foods seasoning prepared by mixing the extract with one or more of salt, saccharides such as sugar, organic acids such as vinegar, MSG (monosodium glutamate), IN (sodium 5'-inosinate), soy sauce, soybean paste, and proteinous hydrolyte with acid or enzyme. There is no limitation on a form of the seasoning, including powder,

paste, liquid and the like. Powdered fat and oil may be mixed with the seasoning. The powdered fat and oil may be supplemented with various known auxiliaries such as the thickening agent, or be emulsified with the emulsifier. Powdering may be done by any method known to those skilled in the art such as spray-dry or freeze-dry. The thus prepared seasoning may be added to foods so as to enhance their body taste.

(3) Processed egg food

[0048] The addition of the long-chain highly unsaturated fatty acid and/or the ester thereof to the processed egg food will enhance its egg flavor. For example, it may provide commercial products such as freeze-dry egg soup with such an excellent egg flavor as it is felt just after a normal cooking. The egg flavor of mayonnaise may be also enhanced. Especially, provision of body taste and enhancement of egg flavor may be realized in low calorie -type mayonnaise with a reduced amount of fat and oil, which has disadvantage of being inferior to normal one in body taste and egg flavor, by adding thereto the long -chain highly unsaturated fatty acid and/or the ester thereof. The long-chain highly unsaturated fatty acid and/or the ester thereof may be added to the freeze-dry egg soup at an eating tim e usually in an amount of 1~1,000 ppm, preferably of 10~1,000 ppm as of the long-chain highly unsaturated fatty acid. It may be added to mayonnaise usually in an amount of 10~1,000 ppm, preferably of 20 ~400 ppm.

(4) Soup

[0049] The body taste of liquid foods such as soup will be enhanced by the addition of the long-chain highly unsaturated fatty acid and/or the ester thereof at an eating time preferably in an amount of 1~2,000 ppm, more preferably of 1~1,000 ppm as of the long-chain highly unsaturated fatty acid.

(5) Curry roux or stew

[0050] The curry roux or stew will be provided with body taste by the addition of the long-chain highly unsaturated fatty acid and/or the ester thereof, which may be mixed with vegetable fat and oil to adjust the curry roux, at an eating time preferably in an amount of 10~2,000 ppm, more preferably of 50~1,000 ppm as of the long-chain highly unsaturated fatty acid. The long-chain highly unsaturated fatty acid and/or the ester thereof may be also used in the curry roux in a form of seasoning.

(6) Japanese soup or its stock

[0051] The addition of the long-chain highly unsaturated fatty acid and/or the ester thereof will inhibit the heat-browning odor of Japanese soup or its stock or will provide it with Japanese soup flavor. Although the long-chain highly unsaturated fatty acid and/or the ester thereof may be added to a final product of Japanese soup, it will show more effectively its advantages by being mixed with the stock of Japanese soup and/or soy sauce during the production processes of the soup. Even after removal of the long-chain highly unsaturated fatty acid and/or the ester thereof once it was mixed with the Japanese soup and heated, its effects will be maintained. The concentration of the long-chain highly unsaturated fatty acid and/or the ester thereof, at an eating time is usually 0.1~500 ppm, preferably 0.1~100 ppm as of the long-chain highly unsaturated fatty acid.

(7) Processed animal meat foods

[0052] The addition of the long-chain highly unsaturated fatty acid and/or the ester thereof will enhance the body taste of dry foods such as burger, meat ball, Chinese-style pork dumplings, steamed Chinese-style pork dumplings, ham and sausage preferably in amount of 20~2,500 ppm, more preferably of 20~1,000 ppm. The addition of the long-chain highly unsaturated fatty acid and/or the ester thereof will inhibit odor derived from vegetable protein and enhance the taste and flavor of the foods utilizing vegetable protein.

(8) Fried rice;

[0053] The use of the long-chain highly unsaturated fatty acid and/or the ester thereof in frying oil will provide the fried rice with body taste and enhance its egg flavor preferably in amount of 2~5,000 ppm, more preferably of 10~1,000 ppm as of the long-chain highly unsaturated fatty acid. The long-chain highly unsaturated fatty acid and/or the ester thereof may be added to frying oil at any stage of cooking, being preferably admixed with the oil though.

(9) Vegetable protein

[0054] The addition of the long-chain highly unsaturated fatty acid and/or the ester thereof to processed foods using vegetable protein, especially granulated soybean protein will mask "top smell" peculiar to the vegetable protein and enhance the taste of the processed foods. The processed foods include burger, meatball, Chinese-style pork dumplings, steamed Chinese-style pork dumplings, ham and sausage, which utilize the soybean protein. In order to show the masking effect of the long-chain highly unsaturated fatty acid and/or the ester thereof, it may be appropriately present in the foods in an amount of 1 ~2,500 ppm, preferably of 1~500 ppm, more preferably of 1~100 ppm as of the long-chain highly unsaturated fatty acid.

(10) Fat and oil for oil cooking and flavoring

[0055] Fat and oil for oil cooking and flavoring may be easily prepared by any suitable methods such as adding or mixing the long -chain highly unsaturated fatty acid and/or the ester thereof to or with vegetable fat and oil.

[0056] Any vegetable fat and oil known to those skilled in the art may be used in the present invention, including soybean oil, rape-seed oil, corn oil, sunflower oil, safflower oil, rice oil, sesame oil, olive oil and palm oil. Among them, the advantages of the present invention will be effectively obtained by adding the body taste enhancer to soybean oil, rape-seed oil, corn oil and palm oil, which are mainly used for heat-cooking such as frying and stir-fry.

[0057] Furthermore, if the vegetable fat and oil contains an isolated trans-isomer in an amount of 10 - 85 %, especially of 20 - 60 %, the advantages of the present invention will be more increased. A content of the isolated trans-isomer expressed as "%" is obtained according to Standard Methods for the Analysis of Fats, Oils and Related Materials by measuring infrared spectrum of methyl ester of a sample fatty acid and determining a percentage of the amount of methyl ester of elaidic acid against the sample. The vegetable fat and oil containing such isolated trans-isomer may be prepared by any method known to those skilled in the art. For example, i t may be prepared by optionally hydrogenating material vegetable fat and oil by any method known to those skilled in the art. The resulting hydrogenated oil may be mixed with non-hydrogenated one.

[0058] The phrase "isolated trans-isomer" in the present specification means an unsaturated fatty acid having an isolated double bond in a trans-form (single double bond or non-conjugated double bond), its content is determined by Standard Methods for the Analysis of Fats, Oils and Related Materials 2.4.4.2-1996.

[0059] In order to obtain the fat and oil composition for oil cooking such as deep - frying or frying, a content of the long-chain highly unsaturated fatty acid in the vegetable fat and oil composition should be usually10 - 100,000 ppm, preferably 10 - 10,000 ppm, more preferably 10 - 8,000 ppm, much more preferably 10 - 3,000 ppm, most preferably 20 - 1,000 ppm. Thus, it is important that the vegetable fat and oil composition of the present invention should have a particular range of the content of the long-chain highly unsaturated fatty acid and/or the ester thereof.

[0060] The thus obtained vegetable fat and oil composition may be used in various heat-cooking methods such as deep-frying and frying, for example, heating preferably at 80°C ~300°C, and more preferably at 110°C ~300°C. The heating treatment at such temperature range will enhance body taste and increase the original tastes of the foods cooked with the vegetable fat and oil composition according to the present invention.

[0061] The fat and oil composition made of the long-chain highly unsaturated fatty acid and/or the ester thereof and vegetable oil may be used as a starting material for oil for seasoning and flavoring having body taste in a usual production process. For example, the oil for seasoning and flavoring may be prepared by mixing the long-chain highly unsaturated fatty acid and/or the ester thereof with one or more of vegetables such as green onion, ginger, garlic, and onion; fish and shells; animal meat; amino acids such as sodium glutamate, followed by heating. It may alternatively be prepared by mixing the fat and oil composition made of the long-chain highly unsaturated fatty acid and/or the ester thereof and vegetable oil with animal meat flavor such as pork, beef and chicken, or butter flavor. The content of the long-chain highly unsaturated fatty acid and/or the ester thereof is usually 0.01~10 %, preferably 0.05~5 % in the vegetable fat and oil composition in order to effectively obtain the advantages of the present invention.

[0062] The present invention will be explained in more detail with reference to the following examples, which should not be construed as limiting a technical scope of the present invention. The term "%" in the following examples mean "% by weight" unless otherwise noted

[0063] Long-chain highly unsaturated fatty acid used in the Examples:

Arachidonic acid (AA): 98% purity, distributed by Wako Chemicals Ltd. and manufactured by ICN;
AA-containing triglyceride (AATG): AA content of 40-45 %, distributed by Nakarai Tesk Ltd. and manufactured by Suntory Ltd;
Borage oil :γ-Linolenic acid content of 20%, manufactured by Statfold Co.;
γ-Linolenic acid: 99% purity, manufactured by SIGMA Co.;
DHA27G : DHA content of 27%, manufactured by NIPPON CHEMICAL FEED CO., LTD.;

DHA: 98% purity, manufactured by SIGMA Co.;
Pure light oil (PL oil): low α-linolenic acid-containing rape seed oil, manufactured by Ajinomoto Co., Inc.

Sensory test:

[0064] In the sensor test, the advantages of the present invention to enhance or make better flavor or taste of the food refer to the increase at least one of "strength of aroma", "strength of flavor", "strength of taste" and "strength of aftertaste" without any deterioration of corresponding "goodness of aroma", "goodness of flavor", "goodness of taste" and "goodness of aftertaste", respectively. Further, the sensor test is also based on the "effects of inhibiting heat-browning odor" and "strength of flavor of Japanese soup 's stock " in Example 10, and on the "strength of soybean proteinous odor" in Example 12. In the sensory test of animal meat extract, aroma is evaluated with a top note of solution, and the flavor is evaluated based on smell or odor that panelists will feel through their nose when they put the solution into their mouth. The strength and goodness of aroma and flavor are evaluated based on good animal smell peculiar to the animal meat extract and on mellow aroma and flavor of the fat and oil.

Panelists: n=7

[0065] The symbols used in the following Tables showing the test results means as follows:

"X": weaker or worse than control;
"Δ": the same degree as control;
"○": stronger or better than control;
"◎": much stronger or much better than control.

Example 1

[0066] Pork extract A-4191 (Ariake Japan Co., Ltd.) was diluted with water or hot water to a concentration of 2 %. AATG was added to the solution at a concentration of 0.001% ~ 0.05% and heated at 95°C~100°C for 2 hours. The sensory test was done in taking the pork flavor and the body taste of oil into account. Non-added extract was used as a control.

[TABLE 1]

Evaluation Item	Cont.	0.001 %	0.002 %	0.005 %	0.01%	0.02%	0.05%
Strength of aroma	-	Δ	○	◎	◎	◎	◎
Goodness of aroma	-	Δ	◎	◎	○	Δ	X
Strength of flavor	-	Δ	○	◎	◎	◎	◎
Goodness of flavor	-	Δ	◎	◎	○	Δ	X
Strength of taste	-	Δ	○	○	○	○	○
Goodness of taste	-	Δ	◎	◎	○	Δ	X
Strength of aftertaste	-	Δ	○	○	○	○	○
Goodness of aftertaste	-	Δ	◎	◎	○	Δ	X

Example 2

[0067] Pork extract A-4191 (Ariake Japan Co., Ltd.) was diluted with water or hot water to a concentration of 2 %. AATG was added to the solution at a concentration of 0.005% and heated at 95°C~100°C for 1~4 hours. The sensory test was done in taking the pork flavor and the body taste of oil into account. Non-heated extract was used as a control.

[TABLE 2]

Evaluation Item	Cont.	1h	2h	3h	4h
Strength of aroma	-	Δ	◎	◎	◎
Goodness of aroma	-	○	◎	◎	○

(continued)

Evaluation Item	Cont.	1h	2h	3h	4h
Strength of flavor	-	Δ	◎	◎	◎
Goodness of flavor	-	○	◎	◎	○
Strength of taste	-	Δ	○	○	○
Goodness of taste	-	○	◎	◎	○
Strength of aftertaste	-	Δ	○	○	○
Goodness of aftertaste	-	○	◎	◎	○

Example 3

[0068] Pork extract powder 2151C (Semba Co., Ltd.) was diluted with water or hot water to a concentration of 40 %. AATG was added to the solution at a concentration of 2% and heated at 95°C~100°C for 2 hours to give "AA pork extract." One pack of powder soup enclosed with ("Sapporo Ichiban Buta Tonkatsu" manufactured by Sanyo Foods Co. Ltd.) was diluted with hot water (500 g), mixed with AA pork extract of a concentration of 0.01~0.07% and used in the test. Non-heated extract (0.07%) was used as a control. The effect of the AA pork extract and taste of soup prepared with the extract were observed.

[TABLE 3]

Evaluation Item	Cont.	0.01 %	0.025%	0.05%	0.07%
Strength of aroma	-	○	◎	◎	◎
Goodness of aroma	-	○	◎	◎	○
Strength of flavor	-	○	◎	◎	◎
Goodness of flavor	-	○	◎	◎	○
Strength of taste	-	○	○	◎	○
Goodness of taste	-	○	◎	◎	○
Strength of aftertaste	-	○	○	◎	○
Goodness of aftertaste	-	○	◎	◎	○

Example 4

[0069] Chicken extract 3943 (IDF) was diluted with water or hot water to a concentration of 2 %. AATG was added to the solution at a concentration of 0.001~0.05% and heated at 95°C~100°C for 2 hours. The sensory test was done in taking the chicken flavor and the body taste of oil into account. Non-added extract was used as a control.

[TABLE 4]

Evaluation Item	Cont.	0.001 %	0.002 %	0.005 %	0.01%	0.02%	0.05%
Strength of aroma	-	○	○	◎	◎	◎	◎
Goodness of aroma	-	○	◎	◎	○	Δ	X
Strength of flavor	-	○	○	◎	◎	◎	◎
Goodness of flavor	-	○	◎	◎	○	Δ	X
Strength of taste	-	○	○	○	○	○	○
Goodness of taste	-	○	◎	◎	○	Δ	X
Strength of aftertaste	-	○	○	○	○	○	○
Goodness of aftertaste	-	○	◎	◎	○	Δ	X

Example 5

[0070] Chicken extract 3943 (IDF) was diluted with water or hot water to a concentration of 2 %. AATG was added to the solution at a concentration of 0.05% and heated at 95°C~100°C for 1~4 hours. The sensory test was done in taking the chicken flavor and the body taste of oil into account. Non-heated extract was used as a control.

[TABLE 5]

Evaluation Item	Cont.	1h	2h	3h	4h
Strength of aroma	-	○	◎	◎	◎
Goodness of aroma	-	○	◎	○	△
Strength of flavor	-	○	◎	◎	◎
Goodness of flavor	-	○	◎	○	△
Strength of taste	-	○	○	○	○
Goodness of taste	-	○	◎	◎	○
Strength of aftertaste	-	○	○	○	○
Goodness of aftertaste	-	○	◎	◎	○

Example 6

[0071] Yeast extract (Gistex XII) was diluted with water or hot water to a concentration of 2 %. AATG was added to the solution at a concentration of 0.001~0.05%. Non-added extract was used as a control.

[TABLE 6]

Evaluation Item	Cont.	0.001 %	0.002 %	0.005 %	0.01%	0.02%	0.05%
Strength of aroma	-	○	○	◎	◎	◎	◎
Goodness of aroma	-	○	◎	◎	○	△	X
Strength of flavor	-	○	○	◎	◎	◎	◎
Goodness of flavor	-	○	◎	◎	○	△	X
Strength of taste	-	○	○	○	◎	○	○
Goodness of taste	-	○	◎	◎	○	△	X
Strength of aftertaste	-	○	○	◎	◎	○	○
Goodness of aftertaste	-	○	◎	◎	○	△	X

Example 7

[0072] One piece of freeze-dry egg soup (Knorr Foods Co. Ltd.) was mixed with hot water (160 g). The solution was then mixed with AATG to a concentration of 0.005 %, or with borage oil to a concentration of 0.05 %, heated at 95 °C~100°C for 1 hour. The sensory test was done in taking the aroma and flavor of egg into account. Non-added soup was used as a control.

[TABLE 7]

Evaluation Item	Cont.	Addition of AATG	Addition of Borage oil
Strength of egg aroma	-	○	○
Goodness of egg aroma	-	○	○
Strength of egg flavor	-	○	○
Goodness of egg flavor	-	○	○

(continued)

Evaluation Item	Cont.	Addition of AATG	Addition of Borage oil
Strength of total taste	-	◎	○
Goodness of total taste	-	○	○
Strength of aftertaste	-	◎	○
Goodness of aftertaste	-	○	○

Example 8

[0073] One pack (17 g) of thick soup (Knorr Cup Soup: Ajinomoto Co. Inc.) was mixed with borage oil (0.5-2.5 g) and then with hot water (150 ml), which was subjected to the sensory test. When the amount of γ-linolenic acid added to the soup is over 3,000 ppm, the aroma will be too strong an d not favorable

[TABLE 8]

Addition of borage oil (g)	0	0.5	1.5	2.5
Concentration of γ-linolenic acid (ppm) at an eating time	-	600	800	3,000
Strength of aroma	-	○	◎	◎
Goodness of aroma	-	◎	◎	X
Strength of flavor	-	○	◎	◎
Goodness of flavor	-	◎	◎	X
Strength of taste	-	○	○	◎
Goodness of taste	-	◎	◎	○
Strength of aftertaste	-	○	○	◎
Goodness of aftertaste	-	◎	◎	○

Example 9

[0074] AATG was added to salad oil manufactured by Ajinomoto Co. Inc. to a final concentration of 1 %, respectively, and a part of which was then heated at 200°C for 5 min. The resulting oil composition was added to mayonnaise manufactured by Ajinomoto Co. Inc. ("Pure Select Half) to a final concentration of 1%. The sensory test was done in taking the aroma and flavor of egg into account. Non-added product was used as a control.

[TABLE 9]

Evaluation Item	Cont.	Addition of AATG	Addition of AATG with heating
Strength of egg aroma	-	○	○
Goodness of egg aroma	-	○	○
Strength of egg flavor	-	○	○
Goodness of egg flavor	-	○	○
Strength of total taste	-	○	◎
Goodness of total taste	-	○	○
Strength of aftertaste	-	○	◎
Goodness of aftertaste	-	○	○

Example 10

[0075] Noodle soup composition was prepared having formulation of TABLE 10. The long-chain highly unsaturated

fatty acid (AATG or DHA27G) and/or the ester thereof was added to the thus obtained concentrate-type noodle soup to a final concentration of 0.0001 ~0.05% and heated at 90 °C for 30 m in. After heating, fat and oil fraction was removed by filtration and the resulting filtrate was diluted three times for evaluation.

[TABLE 10]

Materials	Formulation (g)	Formulation Ratio (%)
Strong soy sauce (KIKKOMAN Co.)	150	50.0
Sugar	27	9.0
Liquid sugar (SANFURAKUTO Co.)	27	9.0
Salt	6	2.0
Soup extracted from bonito	90	30.0
Total	300	100

[TABLE 11]

	No addition	AA	AATG	AATG	AATG	AATG
Added Amount (ppm)	0	0.0003 %	0.0001 %	0.005 %	0.05%	0.5%
Conc. at an eating time	-	1	0.12	6.7	67	670
Strength of aroma	-	○	○	○	◎	◎
Goodness of aroma	-	○	○	◎	○	X
Strength of flavor	-	○	○	○	◎	◎
Goodness of flavor	-	○	○	◎	○	X
Strength of taste	-	○	○	○	○	◎
Goodness of taste	-	○	○	◎	○	X
Inhibiting effect of heat-browning odor	-	◎	○	◎	◎	◎
Strength of Japanese soup flavor	-	◎	○	◎	◎	◎

[TABLE 12]

	No addition	DHA	DHA2 7	DHA2 7	DHA2 7
Added Amount	0	0.0003 %	0.0005 %	0.005 %	0.05%
Conc. at an eating time (ppm)	-	1	0.5	5	50
Strength of aroma	-	○	○	○	○
Goodness of aroma	-	○	○	○	◎
Strength of flavor	-	○	○	○	○
Goodness of flavor	-	○	○	○	◎
Strength of taste	-	○	○	○	○
Goodness of taste	-	○	○	○	◎
Inhibiting effect of heat-browning odor	-	◎	○	◎	◎
Strength of Japanese soup flavor	-	◎	○	◎	◎

[0076] The results in TABLEs 11 and 12 confirmed that the addition of the long-chain highly unsaturated fatty acid and/or the ester thereof would provide the noodle soup with the body taste and Japanese soup flavor, and prohibit the

heat-browning odor.

Example 11

[0077] Strong soy sauce (KIKKOMAN Co.) was diluted with hot water to a concentration of 2 %. AATG was added to the solution at a concentration of 0.001 ~0.05%. Non-added extract was used as a control.

[TABLE 13]

Evaluation Item	Cont.	0.001 %	0.002 %	0.005 %	0.01%	0.02%	0.05%
Strength of aroma	-	○	○	◎	◎	◎	◎
Goodness of aroma	-	○	◎	◎	○	Δ	X
Strength of flavor	-	○	○	◎	◎	◎	◎
Goodness of flavor	-	○	◎	◎	○	Δ	X
Strength of taste	-	○	◎	◎	○	○	○
Goodness of taste	-	○	◎	◎	○	Δ	X
Strength of aftertaste	-	○	○	○	○	○	○
Goodness of aftertaste	-	○	◎	◎	○	Δ	X

Example 12

[0078] Burger was prepared by using the following preparation oil.

(1) AATG (AA content of 40%)
(2) 10% AATG/PL oil (AA content of 4%)
(3) 10% DHA27G/PL oil (DHA content of 2.7%)

[0079] The formulation of the TABLE 14 was mixed by a hobart mixer, divided into a piece of 160 g each, mixed with each preparation oil, shaped into a piece of 30 g each and baked on a hot plate.
Baking conditions: temperature of a hot plate of 200°C, 5 min. for each face x 2 (10 min. in total)

[TABLE 14]

	Formulation (g)	Formulation ratio (%)
Minced low-fat pork	400	39
Lard	50	5
Starch	10	1
Bread crumb	70	7
Onion	160	16
Salt	5	0.5
Sugar	10	1
Soy sauce	10	1
White pepper	1	0.1
Egg white	50	5
Granulated soybean protein	40	4
Water	210	21
Total	1016	100

[TABLE 15]

Preparation oil added	PL oil (cont.)	(2)	(1)	(3)	(1)	(1)	(1)
Amount of Addition (g)	0.2	0.2	0.2	0.3	0.5	1	2
Conc. (ppm) of highly unsaturated fatty acid	0	50	500	34	1250	2500	5000
at an eating time							
Strength of aroma	-	○	◎	○	◎	◎	◎
Goodness of aroma	-	○	◎	○	○	○	X
Strength of flavor	-	○	◎	○	◎	◎	◎
Goodness of flavor	-	○	◎	○	○	○	X
Strength of taste	-	○	◎	○	◎	◎	◎
Goodness of taste	-	○	◎	○	○	○	X
Strength of soybean protein smell	-	X	X	X	X	X	X

[0080] In the above TABLE, "X" in the column "Strength of soybean protein smell" means that the soybean protein smell is weaker than that of the control or not felt.

[0081] The results in TABLE 15 demonstrated that the addition of the highly unsaturated fatty acid in an amount of 50~2,500 ppm followed by heating would provide the burger with the good body taste, without causing any soybean protein smell. On the other hand, over-addition of the same acid would cause unpleasant flavor.

Example 13

< Sausage >

[0082] Wiener sausage was prepared according to a standard method by adding 10% AATG/PI oil (AA content of 4%) to a final concentration of 2~0.1%, or AATG to a final concentration of 1.0% to the following base composition, and subjected to the sensory test.

Formulation ratio:

[0083] Minced pork red meat: 45
Pork back oil: 23
Water with ice: 24
Powdered soybean protein fraction ("AJIPURON SU" Ajinomoto Co. Inc.): 3
Casein sodium: 1
Salt: 1.5
Sugar: 1.5
"Polygon M" (phosphate salt formulation manufactured by Chiyoda Chemical Co. Ltd.,): 0.3
Nitrite Sodium: 0.02
Ascorbic acid sodium: 0.08
White pepper: 0.2
Nutmeg seed: 0.2
Cilantro leaf: 0.1
AA-containing oil: 2.0

Preparation method:

[0084] Materials were cut and mixed well with a food cutter, packed into sheep sausage, and dried, smoked and boiled well in a small smoke house, followed by cooling. The sensory test was carried out on the next day.

[TABLE 16]

		10% AATG	10% AATG	10% AATG 27	10% AATG	AATG
	No addition	0.1 %	0.5 %	1.0 %	2.0 %	1.0 %
AA Concentration (ppm)	-	40	200	400	800	4000
Strength of aroma	-	Δ	○	◎	◎	◎
Goodness of aroma	-	Δ	◎	◎	○	X
Strength of flavor	-	Δ	○	◎	◎	◎
Goodness of flavor	-	Δ	◎	◎	○	X
Strength of taste	-	Δ	○	○	○	◎
Goodness of taste	-	Δ	◎	◎	○	X
Strength of aftertaste	-	Δ	○	○	○	◎
Goodness of aftertaste	-	Δ	◎	◎	○	X
Strength of soybean protein smell		X	X	X	X	X

Example 14

<Curry roux>

[0085] AATG of 0.02~10.0g or borage oil of 5g was added to PL oil to a final volume of 15g. The resulting oil was mixed with soft flour (15g), heated at 120°C for 30 min., then mixed with 3g of curry powder ("Tokusei SB Curry" manufactured by S & B Food Inc.), and heated again at 120°C for 10 min. to give curry roux. Consomme soup (Consomme" manufacture by Ajinomoto Co. Inc., 1.7 % solution) of 500 ml was added and heated. The curry roux was diluted with the consomme soup to give curry soup.

[TABLE 17]

	AATG	AATG	AATG	AATG	AATG	Borage
Added Amount (g)	0	0.02	0.1	1.0	10.0	5.0
Conc. at an eating time (ppm)	0	15	75	750	7500	1875
Strength of aroma	-	○	◎	◎	◎	○
Goodness of aroma	-	○	◎	◎	X	◎
Strength of flavor	-	○	○	◎	◎	○
Goodness of flavor	-	○	◎	◎	X	◎
Strength of taste	-	○	○	◎	◎	○
Goodness of taste	-	○	◎	◎	○	◎

[0086] It was confirmed that the curry roux with the body taste was cooked by using fat and oil containing the long-chain highly unsaturated fatty acid and/or the ester thereof.

Example 15

<Pork cutlet>

[0087] Pork cutlet was deep-fried in vegetable fat and oil preparations comprising the following long-chain highly unsaturated fatty acid and/or the ester thereof.

(1) PL oil

(2) 0.13% AATG/PL oil (AA content of 500 ppm)
(3) 1.25% AATG/PL oil (AA content of 5000 ppm)
(4) 3% AATG/PL oil (AA content of 12,000 ppm)
(5) 5% borage oil/PL oil (γ-linolenic acid content of 10,000 ppm)

[0088] Cutlet was prepared by seasoning pork loin (75g) with salt (0.6g) and a small amount of pepper, coating it with wheat flour, 20% egg solution and bread crumb, followed by deep-frying at 180°C for 30 min. in the above vegetable fat and oil preparations.

[TABLE 18]

	(1)	(2)	(3)	(4)	(5)
Conc. In oil (ppm)		500	5000	12000	10000
Strength of aroma	-	◎	◎	◎	◎
Goodness of aroma	-	◎	◎	X	◎
Strength of flavor	-	◎	◎	◎	◎
Goodness of flavor	-	◎	◎	X	◎
Strength of taste	-	◎	◎	◎	◎
Goodness of taste	-	◎	◎	X	◎

[0089] It was confirmed that the cutlet with the body taste was cooked by frying them in the fat and oil composition containing the long-chain highly unsaturated fatty acid and/or the ester thereof. Over-addition of the long-chain highly unsaturated fatty acid and/or the ester thereof would deteriorate the taste.

Example 16

< Fried rice>

[0090] Beaten egg (100g), rice (500g) and other ingredients (green onion, char siu, etc.) was fried in sequence in a pan coated on its surface with Ajinomoto salad oil (25g) containing AATG (1%) or borage oil (3%), and finally seasoned with salt, pepper and MSG. Fried rice cooked by using only salad oil was considered as a control. The sensory test was carried out in view of the flavor of fried egg and the body taste of oil.

[TABLE 19]

	Control	Addition of AATG	Addition of borage oil
Strength of aroma	-	◎	○
Goodness of aroma	-	◎	○
Strength of flavor	-	◎	○
Goodness of flavor	-	◎	◎
Strength of taste	-	◎	○
Goodness of taste	-	◎	◎
Strength of after taste		◎	○
Goodness of after taste		◎	◎

Example 17

<Fat and oil for flavoring>

[0091] Fat and oil for flavoring was prepared by using the following fat and oil preparation.

< Fat and oil preparation>

[0092]

(1) PL oil
(2) 0.01 % AATG/PL oil (AA content of 0.004%)
(3) 0.1 % AATG/PL oil (AA content of 0.04%)
(4) 1% AATG/PL oil (AA content of 0.4%)
(5) 10% AATG/PL oil (AA content of 4%)
(6) 30% AATG/PL oil (AA content of 12%)

< Preparation of fat and oil for flavoring>

[0093] The above fat and oil preparation (1 L) was heated to 120°C. After being mixed with finely chopped green onion (400g), the fat and oil preparation was stirred while being kept at 100°C until water evaporated completely from the green onion. The green onion was removed by filtration from the fat and oil composition to give oil for flavoring.

<Sensory test of the oil for flavoring>

[0094] Instant noodles ("Sapporo Ichiban Shoyu-Aji" manufactured by Sanyo Foods Co. Ltd) were cooked in accordance with a manufacturer's instruction. The oil for flavoring (3g) was added to one portion of the cooked instant noodles and evaluated.

[TABLE 20]

	(1)	(2)	(3)	(4)	(5)	(6)
Strength of aroma	-	O	O	◎	◎	◎
Goodness of aroma	-	O	O	◎	◎	X
Strength of flavor	-	O	◎	◎	◎	◎
Goodness of flavor	-	O	O	◎	◎	X
Strength of taste	-	O	O	◎	◎	◎
Goodness of taste	-	O	O	◎	◎	X
Strength of aftertaste	-	O	O	◎	◎	◎
Goodness of aftertaste	-	O	O	◎	◎	X

[0095] It was confirmed that the oil for flavoring with the body taste was prepared by using the fat and oil composition made of the long -chain highly unsaturated fatty acid and/or the ester thereof and the vegetable oil. Over-addition of the long-chain highly unsaturated fatty acid and/or the ester thereof would deteriorate the taste.

Advantages of the invention

[0096] It has been confirmed that the body taste of foods can be enhanced and the original tastes of foods can be increased when the foods are mixed with the long-chain highly unsaturated fatty acid and/or the ester thereof or the vegetable fat and oil composition comprising a predetermined amount of the long-chain highly unsaturated fatty acid and/or the ester thereof, or when the foods are heated and cooked with the same vegetable fat and oil composition.

Claims

1. A method for making taste of seasoning better, and/or for providing seasoning with body taste, comprising adding a long-chain highly unsaturated fatty acid and/or an ester thereof to the seasoning.

2. A method for enhancing body taste of extract, comprising adding a long-chain highly unsaturated fatty acid and/or an ester thereof to the extract.

3. A method for enhancing egg flavor of processed egg food, comprising adding a long-chain highly unsaturated fatty acid and/or an ester thereof to the processed egg food.

4. A method for enhancing body taste of soup, comprising adding a long -chain highly unsaturated fatty acid and/or an ester thereof to the soup.

5. A method for providing curry roux or stew with body taste, comprising adding a long-chain highly unsaturated fatty acid and/or an ester thereof to the curry roux or stew.

6. A method for inhibiting heat-browning odor of Japanese soup or its stock, comprising adding a long-chain highly unsaturated fatty acid and/or an ester thereof to the Japanese soup or its stock.

7. A method for providing processed animal meat food with body taste, comprising adding a long-chain highly unsaturated fatty acid and/or an ester thereof to the processed animal meat food.

8. A method for enhancing body taste and fried-egg flavor of fried rice, comprising adding a long-chain highly unsaturated fatty acid and/or an ester thereof to the fried rice.

9. A method for inhibiting proteinous odor of vegetable protein, comprising adding a long-chain highly unsaturated fatty acid and/or an ester thereof to the vegetable protein.

10. A method according to any one of Claims 1 - 9, wherein the long-chain highly unsaturated fatty acid is an n-6 long-chain highly unsaturated fatty acid.

11. A method according to Claim 10, wherein the long-chain highly unsaturated fatty acid is arachidonic acid.

12. A method according to any one of Claims 1 - 9, wherein the long-chain highly unsaturated fatty acid is an n-3 long-chain highly unsaturated fatty acid, and is obtainable by oxidization treatment.

13. A method according to any one of Claims 1 - 12, wherein the long-chain highly unsaturated fatty acid is derived from microorganism.

INTERNATIONAL SEARCH REPORT

International application No.
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A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] A23L1/226, A23L1/22, A23L1/32, A23L1/39, A23L1/31, A23L1/015

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] A23L1/22-1/32, A23L1/39, A23L1/015, A23D9/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
JSTPlus (JOIS)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 2002-095439 A (Asahi Denka Kogyo Kabushiki Kaisha), 02 April, 2002 (02.04.02), (Family: none)	1-13
A	JP 8-311485 A (Loders-Croklaan B.V.), 26 November, 1996 (26.11.96), & EP 739589 A1 & DE 69613271 E & US 6410078 B1	1-13
A	JP 2001-226693 A (The Nisshin Oil Mills, Ltd.), 21 August, 2001 (21.08.01), (Family: none)	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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